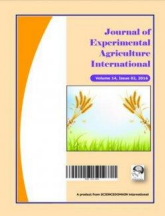

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Effect of Desiccation Time on Seed Moisture and Regeneration of Mangaba (*Hancornia speciosa*) Embryos

Fernanda Vieira Santana[1*], Annie Carolina Araújo de Oliveira[1], Leila Albuquerque Resende de Oliveira[1], Isabella Cícera Dias Miranda[2], Caroline de Araújo Machado[3], Paulo Augusto Almeida Santos[1], Ana Veruska Cruz da Silva[3] and Ana da Silva Ledo[3]

[1]Federal University of Sergipe, Av. Marechal Rondon, s/n - Jd. Rosa Elze, São Cristóvão - SE, 49100-000, Brazil.
[2]Tiradentes University, Av. Murilo Dantas, 300 - Farolândia, Aracaju - SE, 49032-490, Brazil.
[3]Brazilian Agricultural Research Corporation, Embrapa Tabuleiros Costeiros, Av. Beira Mar, 3250 - Jardins, Aracaju - SE, 49025-040, Brazil.

Authors' contributions

This work was carried out in collaboration between all authors. Authors ASL, PAAS and FVS designed the study. Author FVS performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors FVS, ASL, PAAS and AVCS managed the analyses of the study. Authors ACAO, LARO, ICDM and CAM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Hancornia speciosa Gomes, popularly known as mangaba tree, is a fruit tree native to Brazil, with natural occurrence in several regions. However, some factors have contributed to the reduction of natural populations of this species, in addition to the recalcitrant characteristic of its seeds, which hinders their storage for conservation purposes. The application of plant tissue culture techniques is a complementary strategy to the conservation of the existing genetic variability and allows

**Corresponding author: E-mail: nanda.vst@hotmail.com;*

accelerating the multiplication of promising genotypes. This study aimed to evaluate the effect of the desiccation time of mangaba tree on seed moisture and regeneration using cryopreserved and non-cryopreserved embryos. Seeds were extracted from mature and immature fruits of BRA00074140-5 accession, belonging to Mangaba Tree Active Germplasm Bank of Embrapa Tabuleiros Costeiros, Sergipe, Brazil. At 24 hours after seeds extraction, they were desiccated in Magenta ™ boxes containing silica gel (50 g/magenta) for 0, 8, 10, 12, and 14 hours, at room temperature. Seed moisture (four samples of five seeds/maturation) was determined for each desiccation period. After each desiccation period, seeds were inoculated in germination medium (control 2), and two samples of ten seeds for each desiccation time were immediately placed in cryotubes and immersed in liquid nitrogen (-196ºC). For the evaluation of the germination percentage, seeds were thawed in sucrose solution for 24 hours. Subsequently, embryos were excised and inoculated in MS culture medium with 3% sucrose and 0.30% Phytagel® and evaluated at 60 days of culture. Cryopreserved seeds regenerated no embryos. Further studies should be performed to obtain regeneration from cryopreserved seeds.

Keywords: Hancornia speciosa Gomes; conservation; cryopreservation; genetic resources.

1. INTRODUCTION

Hancornia speciosa Gomes, popularly known as mangaba tree, is a fruit species belonging to the Apocynaceae family and Dicotyledoneae class. It presents wide geographic occurrence in Brazil, being found in several regions, from Coastal Tablelands and coastal lowlands of Northeastern Brazil, where it is more abundant, to Midwestern, Northern and Southeastern regions [1].

Mangaba tree fruits present aromatic, sweet pulp, with a characteristic flavour very appreciated in the market, for both fresh consumption and industry. Thus, it is of great importance to the Northeastern region of Brazil, as it provides income and food for thousands of local families. Mangaba is one of the fruits most requested by local industries since its processing results in several products, such as pulps, jellies, ice creams, juices, sweets, cakes, biscuits and liqueurs [2,3].

Due to the socioeconomic importance of this species, the problem of the genetic erosion process of mangaba tree emerges mainly due to its natural occurrence in areas of marked real estate speculation and intense agricultural exploitation [4]. The conservation of mangaba tree germplasm has already been carried out in the field by research institutions and universities, but the vulnerability of accessions in this type of conservation is high, mainly due to the incidence of pests, diseases and climatic variations, which emphasise the importance of the development of complementary conservation techniques, such as *in vitro* germplasm conservation [5,6].

As an alternative to this problem, *in vitro* conservation techniques include cryopreservation, which aims at the conservation of explants for long periods and at ultra-low temperatures, storing the material in liquid nitrogen at temperature of -196ºC, which allows a drastic reduction of the cell metabolism and paralyzes cell division activities, keeping the preserved biological material intact [7]. For this technique to present promising results, it is necessary to avoid the formation of ice crystals during the process, which are more frequent in the cooling and reheating stages [8]. The reduction of the water content in explants to extremely low levels, capable of avoiding the formation of these crystals, can be considered the most critical step in obtaining successful cryopreservation protocols [9].

Vitrification can be achieved by dehydrating tissues to moisture content where there is no free water for crystallisation before submitting samples to liquid nitrogen. Dehydration can be obtained by evaporation of water or by treatment with concentrated chemical cryoprotectant solutions [10]. The drying stage provides a reduction in cell metabolism, which may benefit storage, but in desiccation-sensitive species, such as mangaba, water removal can cause irreversible damage to cells, compromising seed viability [11].

Some authors have already described research works with the cryopreservation technique applied to lateral buds and apical meristems of mangaba tree. Efficient results have been reported with the droplet vitrification and vitrification techniques with more than 70% regeneration of shoot tips after cryopreservation [12]. Using the droplet vitrification technique, the same authors obtained 90% shoot tip growth

resumption. Regarding the techniques of encapsulation-vitrification and droplet vitrification of lateral buds, 89 and 84% regeneration was obtained by [13]. There are no studies in the literature on the cryopreservation of seeds and structures such as zygotic embryos and cotyledonary axes. However, some studies with intermediate species such as jenipapo (*Genipa americana* L.) [14], cotton (*Gossypium hirsutum* L.) [9] and coffee trees (*Coffea arabica* L.) [15] obtained regeneration using embryonic axes.

The present work aimed to evaluate the effect of different dehydration times on silica gel on the regenerative capacity of cryopreserved *Hancornia speciosa* Gomes embryos.

2. MATERIALS AND METHODS

2.1 Plant Material

Seeds extracted from mature ("falling") and green-mature ("almost mature") mangaba fruits of BAG Mangaba BRA00074140-5 accession (12 years old), located at Embrapa Tabuleiros Costeiros, Itaporanga d'Ajuda, Sergipe, Brazil, were used as explants. Seeds were manually pulped and allowed to dry at room temperature for 24 hours in a paper towel to remove excess water.

After drying, the asepsis stage of seeds was performed, initially with washing in Tween 20® solution (1-2 drops) and sterile water for the total removal of pulp and then in laminar flow chamber, being immersed in 70% alcohol for 2 minutes, and then in sodium hypochlorite solution with 2-2.5% active chlorine for 15 minutes. Then, seeds were washed up to the total pulp removal and placed on paper towels for 24 hours at room temperature.

2.2 Effect of Desiccation Time on Moisture and Viability of Mangaba Seeds

To obtain the initial seed germination percentage (control 1), four replicates of five seeds were inoculated in MS culture medium [16] with 3% sucrose and 0.3% Phytagel® (germination medium).

For studies on the effect of desiccation on germination of mangaba embryos, seeds were desiccated on sterile filter paper in autoclaved Magenta ™ flasks (77 mm × 77 mm × 97 mm) containing 50 g of silica gel/flask for 0, 8, 10, 12 and 14 hours at room temperature.

For each desiccation period, three samples of five seeds each were inoculated in the germination medium (control 2) and four samples of 10 seeds each were immediately placed in cryotubes and immersed in liquid nitrogen (-196ºC).

To determine the initial moisture of seeds and after each desiccation period, four replicates of five seeds were weighed to obtain the fresh mass (FM) and transferred to the greenhouse for 24 hours at a temperature of 105 ± 3ºC to obtain the dry mass (DM). Moisture was determined by the following formula [17]:

$$Umidade\ (\%) = \frac{Massa\ fresca - Massa\ seca}{Massa\ fresca} * 100$$

Germination percentage, shoot and root length, and a number of leaves were evaluated at 60 days after inoculation. A completely randomised design (CRD) was used in a 2 x 5 factorial scheme (two maturation types x five desiccation times), with three replicates, the plot being represented by 15 embryos.

2.3 Effect of Desiccation Time on the Regeneration and Growth of Cryopreserved Mangaba Tree Embryos

For studies on the effect of desiccation on germination, seeds were desiccated in Magenta ™ flasks (77 mm × 77 mm × 97 mm) containing 50 g of silica gel for 0, 8, 10, 12 and 14 hours at room temperature.

After each desiccation period, three samples of five seeds were soaked in 0.3 M sucrose solution for 24 hours, and after that period, their embryos were excised and inoculated in germination medium (control 2). Four samples of five seeds were separated, each being immediately placed in cryotubes and immersed in liquid nitrogen (-196 ºC).

To evaluate the germination percentage of embryos after cryopreservation, cryotubes were thawed by immersing seeds in 0.3 M sucrose solution for 24 hours at room temperature. After this time, embryos were excised and immediately inoculated in MS culture medium with 3% sucrose and 0.3% Phytagel®. Cultures were maintained in a growth room with a controlled

temperature of 25±2°C, relative humidity of about 70% and a photoperiod of 12 hours of light with a luminous intensity of 60 $\mu molm^{-2}s^{-1}$.

The growth variables shoot, and root length and number of leaves was evaluated at 60 days of inoculation, and the desiccation time of 14 hours was not considered due to the abnormal development of seedlings. A completely randomised design in a 2 x 4 factorial scheme was used (two maturation types and four desiccation times). For moisture content and germination percentage, a completely randomised design in a 2 x 5 factorial scheme was used (two maturation types and five desiccation times).

2.4 Statistical Analyses

Data were submitted to analysis of variance and compared by the Tukey's test at 5% significance for studies on qualitative effects, and regression equations were estimated for quantitative effects, the SAS statistical software was used [18].

3. RESULTS AND DISCUSSION

3.1 Effect of Desiccation Time on Seed Moisture of Mature and Mature-green Mangaba Fruits

There was an isolated effect of the desiccation time and maturation stage of fruits on seed moisture, with no significant effect of the interaction of factors. Fruit moisture presented linear behavior ($y = -1.0783^{**} x + 38.449$; $R^2 = 0.9855$, Fig. 1). At time T0, moisture was 38.54%, with the exposure to silica gel in different periods, the water content was reduced to 24.24% in 14 hours of desiccation.

The initial and final moisture content presented different values, according to the application of the different desiccation times in silica gel, with average of 10% difference, a considerable value when it comes to cryopreservation. In the case of mangaba tree, its recalcitrant seeds presented high water content, reinforcing the need to reduce moisture in zygotic embryos [19]. When testing direct dehydration in zygotic mangaba tree embryos, the initial moisture of 66% and final 25% after 120 minutes of dehydration were observed [20].

Considering the maturity stage of fruits, it was possible to observe that seeds from mature fruits presented 33.68% moisture, significantly higher than those from mature-green fruits, which reached 24.24% humidity (Table 1).

3.2 Effect of Desiccation Time on the Germination and Growth of Non-cryopreserved Mangaba Zygotic Embryos

There was a significant effect of desiccation time and maturity stage of fruits alone on the germination percentage. There was no significant effect of the interaction among factors. The germination percentage presented negative linear behavior ($y = -4.589^{**} x + 94.384^{**}$, $R^2 = 0.9043$, Fig. 2). At time 0h, it was 90%, reducing to 20% with increasing desiccation time. High germination percentage (92%) of zygotic mangaba tree embryos not submitted to desiccation was also observed by Freire [21]. Using direct dehydration in mangaba tree embryos, 100% germination was observed at the time of 20 minutes; and after 120 minutes of dehydration, germination was dramatically reduced to 33% in studies conducted by Santos [20].

These values are higher than those observed in this study, which indicates that the desiccation of seeds and embryos themselves may present different behaviours according to the technique used. The dehydration of zygotic embryos has as a limitation to the complexity of tissues that make up the embryo and have a differentiated sensitivity to dehydration [22].

Table 1. Average [1] moisture content and germination percentage of mangaba zygotic embryos as a function of the maturity stage of fruits

Fruit stage maturation	Moisture (%)	Germination (%)
Green-mature (almost mature)	24.24b	42.67b
Mature (falling)	33.67a	65.33a
CV (%)	6.00	22.44

[1] *Means followed by the same lowercase letter in the column do not differ from each other by the Tukey test at 5% significance*

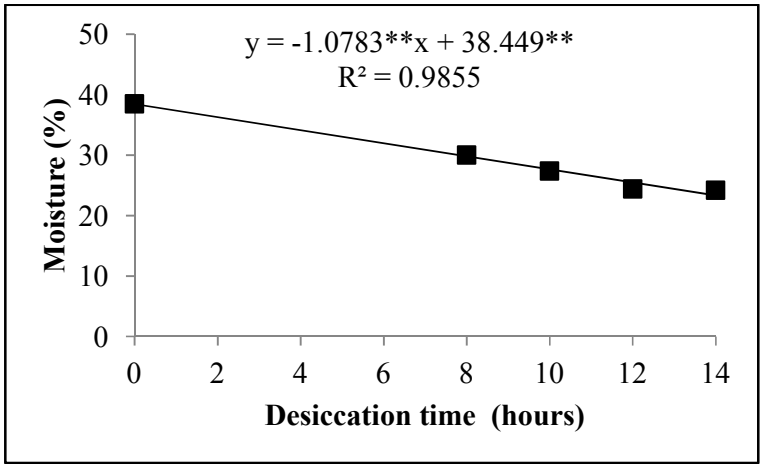

Fig. 1. Effect of desiccation time on the moisture content of mangaba seeds from BRA00074140-5 accession (12 years old)

Considering the maturity stage of fruits (Table 1), it was possible to observe 65.33% germination of zygotic embryos originating from mature (falling) fruits, significantly higher than 42.67% germination of mature green embryos (almost mature).

There was a significant effect of desiccation time, and maturity stage of fruits alone on variables shoot length, root length and number of leaves. There was no significant effect of the interaction among factors. Shoot length showed negative linear behavior (y = -0.1234 ** x + 4.5219 **, R^2 = 0.7165, Fig. 3A). In relation to root length, the regression equation also showed negative linear behavior (y = -0.222 ** ** + 4.3.295 **, R^2 = 0.8492, Fig. 3B). Only in this variable, the maturity stage was not significant for the development of seedlings (Table 2). Variable number of leaves presented polynomial behavior (y = -0.0237x^2 ns - 0.3502x ns + 5.0685 **, R^2 = 0.8322, Fig. 3C), unlike the other variables analyzed.

The reduction in germination percentage, shoot length, root length and number of leaves is directly related to dehydration, since this process promotes deleterious intracellular modifications that affect embryo growth and development [20]. Dehydration causes changes in several organelles such as mitochondria, endoplasmic reticulum and Golgi complex, which compromises embryo metabolism [19]. These are the factors that limit the survival of zygotic embryos of recalcitrant species when submitted to desiccation and freezing in liquid nitrogen [22].

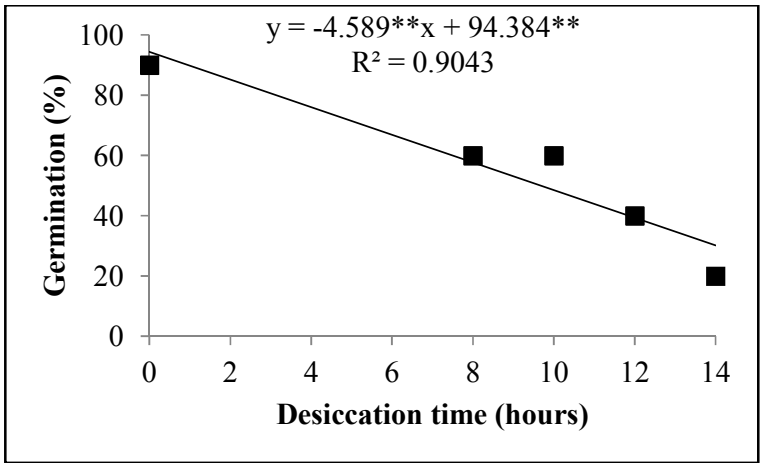

Fig. 2. Effect of desiccation time on the germination of mangaba zygotic embryos

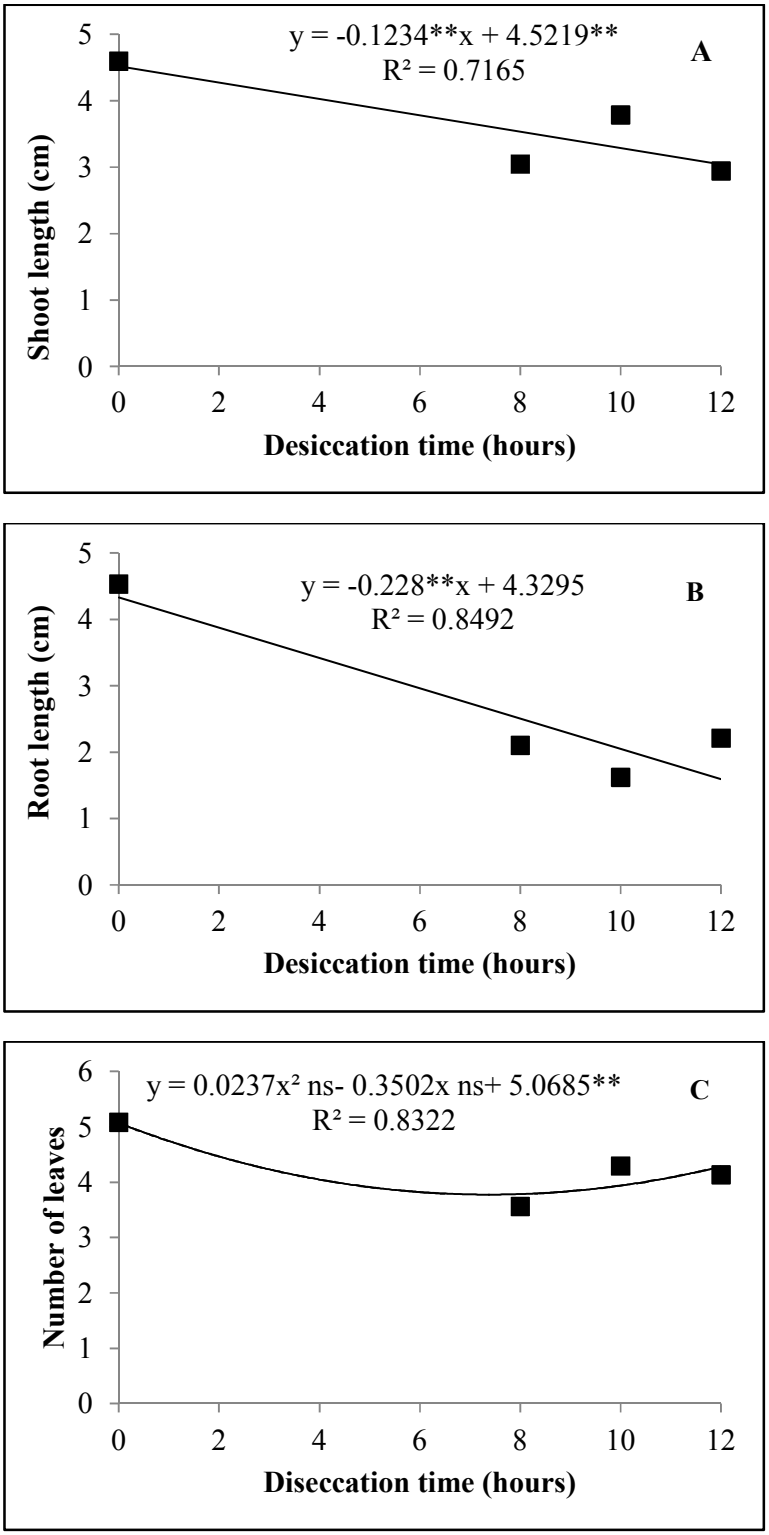

Fig. 3. Effect of desiccation time on the growth of mangaba seedlings obtained from mature (falling) and mature-green fruits (almost mature) at 60 days of *in vitro* culture. A- shoot length; B- root length and C- number of leaves

Table 2. Average[1] shoot length (SL), root length (RL) and number of leaves (NL) of mangaba zygotic embryos as a function of fruit maturity stage

Fruit stage maturation	SL (cm)	RL (cm)	NL
Green-mature (almost mature)	2.97b	2.69a	3.80b
Mature (falling)	5.05a	3.05a	5.34a
CV (%)	34.30	58.06	42.85

[1] *Means followed by the same lowercase letter in the column do not differ from each other by the Tukey test at 5% significance*

Fig. 4. Embryo regeneration and growth of mangaba seedlings obtained from A- mature (falling) and B- green-mature (almost mature) fruits after different desiccation times at 60 days of *in vitro* culture in MS culture medium

Considering the maturity stage of fruits (Table 2), it was possible to observe that the growth and development of seedlings from zygotic embryos of mature fruits (falling) showed a greater increase in variables shoot length and number of leaves, being significantly higher than seedlings from zygotic embryos of mature-green fruits (almost mature).

3.3 Effect of Desiccation Time on the Regeneration and Growth of Cryopreserved Mangaba Tree Embryos

At 60 days of inoculation of cryopreserved mangaba tree zygotic embryos, only one embryo exposed to desiccation for 12 hours emitted the radicle. In contrast, non-cryopreserved embryos at both maturity stages became greenish with germination (Fig. 5A) and showed the formation of normal seedlings at all desiccation times. No oxidation of cryopreserved embryos was observed, which remained whitish (Fig. 5B).

Mangaba cryopreservation is already successfully performed on other types of explants; however, the use of zygotic embryos is still a challenge. In a study that the vitrification technique was applied, exposure of embryos in DMSO and sucrose, no regeneration was observed [23]. A study using desiccation in a laminar flow chamber followed by the osmoconditioned rehydration of zygotic mangaba tree embryos also obtained no satisfactory results [20].

It is important to emphasise that zygotic embryos of recalcitrant species are composed of complex and sensitive tissues, which limits their survival to desiccation and freezing in liquid nitrogen [22]. However, other studies have shown promising results using other recalcitrant species. Authors obtained 98% *in vitro* regeneration of embryonic axes of coffee seeds (*Coffea arabica* L.) with 23% moisture after freezing in liquid nitrogen [16]. Results above 80% after cryopreservation of embryonic axes of two different cotton cultivars (*Gossypium hirsutum* L.) with moisture between 9 and 16% have also been observed [9].

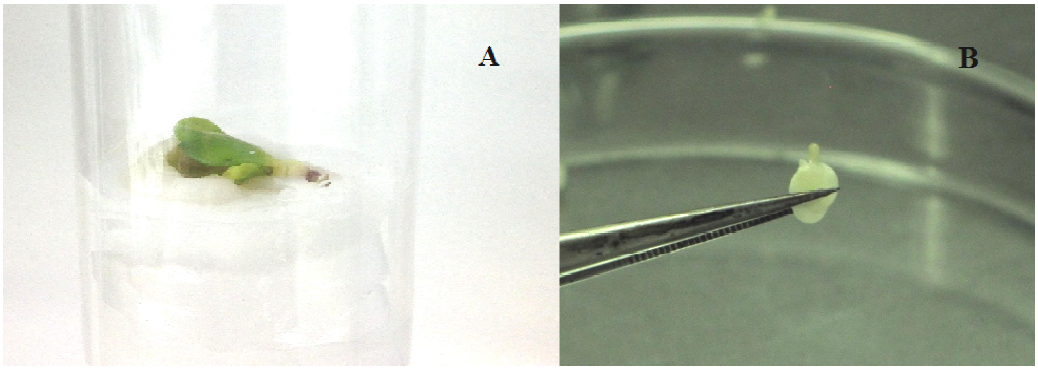

Fig. 5. A- Non-cryopreserved zygotic embryos of mangaba with greenish colouration and beginning of regeneration; B- Cryopreserved zygotic embryos of mangaba with characteristic white colour after excision

Several factors inherent to the embryo can affect cryopreservation, such as the development stage, the species and the production method. Cryopreservation factors such as cryoprotectant type, freezing curve rate and type of physical support for the embryo are other alternatives that influence the maintenance of the viability of embryos submitted to the cryopreservation process [24].

It could be inferred that the methodology must be adapted, since embryos coming from seeds of both maturity stages germinated and formed normal seedlings, which indicates seed viability and regeneration capacity of the species even under desiccation conditions. Thus, there is a hypothesis that something in the cryopreservation process can be modified. One possibility would be to adjust moisture between 10% and 20%, although mangaba seeds lose their power of germination quickly and do not withstand the sudden loss of water, it is possible that the dehydration was not enough to prevent the formation of ice crystals in the intracellular medium, which causes the rupture of the system of cell membranes and consequently, the collapse of cells and their death [25].

4. CONCLUSION

Seed moisture reduces with increasing desiccation times at both maturity stages of fruits.

Seeds from mature fruits have higher water content.

Germination reduces as desiccation time increases.

Zygotic embryos of mature fruits present higher germination percentage and development of seedlings.

Desiccation times affect growth variables of seedlings derived from seeds of mature-green fruits.

The desiccation technique does not promote the survival and regeneration of mangaba zygotic embryos after cryopreservation.

Considering that the species *Hancornia speciosa* Gomes presents recalcitrant seeds, the data obtained in this study will support further studies based on the elucidated factors that affected cryopreservation, allowing new alternatives to be used to establish a satisfactory cryopreservation protocol.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Machado LDL, Ramos MLG, Caldas LS, Vivaldi LJ. Selection of matrices and mangabeira clones for in vitro culture. Pesquisa Agropecuária Brasileira. 2004; 39(5):431-35.
2. Lima ILP, Scariot A. Good management practices for sustainable mangaba extractivism. Brasília, Brazil: Embrapa Genetic Resources and Biotechnology; 2010.
3. Perfect DGA, Carvalho N, Lopes MCM, Schmidt FL Characterization of fruits of mangabas (*Hancornia speciosa* Gomes) and study of pulp extraction processes. Journal of Neotropical Agriculture. 2015; 2(3):1-7.

4. Silva Júnior JF, Mota DM, Schmitz H, Rodrigues RFA. between tabuleiros, restingas and cerrados: The *in situ* conservation of the mangabeira by the traditional communities of extractivists. In: Symposium on Genetic Resources for Latin America and the Caribbean, 10. 2015, Bento Gonçalves. Genetic resources in the 21st century: from Vavilov to Svalbard. Anais. Brazilian Society of Genetic Resources; 2015.
5. Lédo AS, Sá AJ, Santos MC, Silva Júnior JF, Muniz AVCS. Protocol for in vitro conservation by slow growth of mangabeira (*Hancornia speciosa* Gomes). Aracaju, SE: Embrapa Coastal Trails. 2011;4 . (Comunicado Técnico, 115).
6. Silva Júnior, JF, Muniz AVCS, Lédo AS, Maia MCC, Carvalhaes MA, Silva SMC et al. Descriptors for Mangaba (*Hancornia speciosa* Gomes). Bioversity International, Rome, Italy and Brazilian Agriculture Research Corporation, Embrapa Tabuleiros Costeiros, Aracaju, SE, Brazil; 2018.
7. Engelmann F. Plant cryopreservation: Progress and prospects. *In vitro* Cell & Developmental Biology Plant. 2004; 40(5):427-33.
8. Mazur P. Freezing of living cells: Mechanisms and implications. American Journal of Physiology-cell Physiology. 1984;247(3):125-42.
9. Lopes KP, Almeida FAC, Carvalho JMFC, Bruno RLA. Cryopreservation of axes of zygotic cotton embryos. Brazilian Journal of Agricultural and Environmental Engineering. 2013;17(3):291-98.
10. Santos IRI, Solomon AN. Cryopreservation of plant germplasm. Biotechnology, Science & Development. 2001;20:60-65.
11. Kohoma S, Maluf AM, Bilia DAC, Barbedo CJ. Drying and storage of *Eugenia brasiliensis* Lam. ("Grumixameira") seeds. Brazilian Journal of Seeds. 2006;28(1):72-78.
12. Santos PAA, Paiva R, Silva LC, Souza AC, Santana MC, Silva DPC. Cryopreservation of the mangaba tree (*Hancornia speciosa* Gomes): A protocol for long term storage. Acta Scientiarum Agronomy. 2015;37(3): 289-96.
13. Prudent DO, Paiva R, Nery FC, Paiva PDO, Reis MV, Silva LC. Cryopreservation of lateral gemstones of mangabeira: The role of proline. Health & Science Online Magazine. 2014;3(3):86-93.
14. Santos IRI, Salomão AN. Viability assessment of *Genipa americana* L. (Rubiaceae) embryonic axes after cryopreservation using *in vitro* culture. International Journal of Agronomy. 2016:1-6.
15. Pinto MS, Paiva R, Silva DPC, Santos PAA, Freitas RT, Silva LC. Cryopreservation of coffee zygotic embryos: Dehydration and osmotic rehydration. Ciência e Agrotecnologia. 2016;40(4):380-389.
16. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum. 1962;15(3):473-97.
17. Rules for Seed Analysis. Ministry of Agriculture, Livestock and Food Supply. Secretariat of Agricultural Defense Map/ ACS. Brasilia DF; 2009.
18. SAS Institute Inc. SAS/Stat user's guide: Statistics. Version 9.2. Cary, NC.
19. Berjak P, Pammenter NW, Wesley-Smith J. Rate of dehydration, state of subcellular organization and nature of cryoprotection are critical factors contributing to the variable success of cryopreservation: studies on recalcitrant zygotic embryos of *Haemanthus montanus*. Protoplasma. 2012;249(1):171-86.
20. Santos PAA. *In vitro* culture and conservation of *Hancornia speciosa* Gomes. PhD Thesis in Plant Physiology. Post-graduation in Plant Physiology, Federal University of Lavras, Minas Gerais; 2013;94f.
21. Freire KCS, Coelho GG, Russo SL, Silva AVC, Lédo AS, Sá A.J, Machado CA. In vitro germination of zygotic embryos and acclimatization of mangaba seedlings from the embryo culture (*Hancornia speciosa* Gomes). Scientia Plena. 2011;7(11):1-7.
22. Engelmann F. Use of biotechnologies for the conservation of plant biodiversity. In Vitro Cell & Developmental Biology-Plant. 2011;47(4):5-16.
23. SILVA, E. de O. Propagation and storage of mangabeira seeds (*Hancornia speciosa* Gomes). PhD Thesis in Agronomy. Federal University of Paraíba, Areia. 2010; 105f.
24. Santin TR, Blume H, Mondadori RG. Embryo cryopreservation - vitrification methodologies. Veterinary and Animal Science. 2009;16(4):561-74.

25. Santos IRI. Cryopreservation of embryonic axes of citrus species using encapsulation and dehydration. Brasília. Documents - Embrapa Genetic Resources and Biotechnology. 2004; 24.

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